Operating Instructions

Strong anion exchange Chromatography Media
Cellufine™ MAX Q-r, Cellufine™ MAX Q-h

Description

Cellufine MAX is the new name of 2nd generation Cellufine chromatography media.
Cellufine MAX Q strong ion exchangers are highly cross-linked, surface modified media with high dynamic binding capacities and stability at high flow velocities. These high performance optimized media offer significant opportunity for increasing downstream purification throughput.
The characteristics of Cellufine MAX "-**r**" sires are high **r**ecovery, high **r**esolution and **r**obust.
The characteristic of Cellufine MAX "-**h**" is the **h**ighest adsorption capacity in present products (2010).

Characteristics of Cellufine MAX

Type	Cellufine MAX Q-r	Cellufine MAX Q-h
Ion exchange type	Strong Anion / $-N^+(CH_3)_3$	
Matrix	Highly cross-linked cellulose with dextran scaffold	
Particle size	ca. 40 – 130 μm (Average 90)	
Ion exchange capacity	0.10～0.20 meq /ml	0.13～0.22 meq /ml
Flow velocity	600 cm/h(0.3 MPa) I.D.30 cm-L20 cm, pure water at 24 °C	
Dynamic binding capacity	> 110 mg BSA/ml	> 180 mg BSA/ml
pH working range	2 – 12	2－12
pH stability (40°C, 1week)	2－12	2－12
Chemical stability	Stable all commonly used aqueous buffers, 1 M NaOH	
Storage	20 % ethanol	

Packing to the column

1. Calculate volume required of the desired bed dimension.
   (a) Packed bed volume= column cross sectional area (cm^2) x bed height (cm)
   (b) Required sedimented gel volume≒Packed bed volume x 1.2
   (c) The slurry concentration for bottle of Cellufine MAX Q is approximately 50 % in 20 % ethanol.
2. Washing the gel with water or the appropriate buffer.

3. Prepare a 40 – 60 % (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer. Allow to equilibrate at ambient temperature for one hour.
4. Gently stir. If required place under vacuum to degas.
5. Column
   (a) A column is prepared according to the instruction of the column.
   (b) A filter is dipped in a packing solution or 20 % ethanol before use and extracts air.
   (c) Pour the packing solution into the column tube and it checks that the solution comes out from a column exit certainly. A stop plug is shut when approximately 0.5 to 1 cm height of the solution remains.
6. Carefully pour the slurry into the column without air bubbles. Depending on the volume, a filler tube may be necessary.
7. Mount the top adapter on the top of column. (Don't put in air)
8. Open the column outlet and begin pumping elution buffer for 10 min at 1000 cm/h or 0.3Mpa. Caution: do not excess the operation pressure limit for using column.
9. Mark the gel bed height. Stop the pumping and stop plug in column outlet.
10. Disconnect the top adapter line from the pump. The seal is loosened and move the top adapter to mark of the gel bed hit at packing.
11. After the bed stabilizes, lock the adapter and set the line from the pump. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

Packing to the fixed length column

1. Calculate volume required of the desired bed dimension.
   (a) Packed bed volume= column cross sectional area (cm^2) x bed height (cm)
   (b) Required sedimented gel volume=Packed bed volume x 1.15
   (c) Note: When using a packing connector, the amount of excesses can be used from required sedimented gel volume.
   (d) The slurry concentration for bottle of Cellufine MAX Q is approximately 50% in 20% ethanol.
2. Washing the gel with water, 0.1 M NaCl solution or the appropriate buffer.
3. Prepare a 40 – 60 % (v/v) slurry with water, 0.1 M NaCl solution or the appropriate buffer. Allow to equilibrate at ambient temperature for one hour.
4. Gently stir. If required place under vacuum to degas.
5. Column
   (a) A column is prepared according to the instruction of a using column.
   (b) A filter is dipped in a packing solution or 20% ethanol before use and extracts air.
   (c) Pour the packing solution into the column tube and it checks that the solution comes out from a column exit certainly. A stop plug is shut when approximately 0.5 to 1 cm high of the solution remains.

6. Carefully pour the slurry into the column and packing connector without air bubbles. Depending on the volume, a filler tube may be necessary.
7. Mount the top adapter on the top of packing connector.
8. Open the column outlet and begin pumping elution buffer for 10 min at 1000 cm/h or 0.3 MPa. Caution: do not excess the operation pressure limit for using column.
9. Stop the pumping and stop plug in column outlet.
10. Disconnect the top adapter line from the pump. Remove the packing connector. Before remove excess medium from the packing connector, if necessary.
11. Mount the top adapter, lock the adapter and set the line from the pump. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

Evaluation of packing

See appendix 1

Operating Guidelines

General Operation

Typically, adsorption to Cellufine MAX Anion Exchange medium occurs in relatively low ionic strength (e.g., below 0.1 M NaCl) in the pH range from 6 – 9 under the *pI* of the target protein.

The binding capacity is strongly affected by pH and conductivity. Under these conditions, proteins with neutral or net negative charge will bind. Bound components are then resolved by stepwise elution with buffers containing progressively higher salt concentration or by elution with a linear salt gradient.

Sample Preparation and Load

Prepare sample by centrifugation or microfiltration to remove insoluble material. Samples should be prepared to a concentration of 1 – 20 mg/ml, in binding buffer or at comparable conditions of ionic strength and pH. If necessary, exchange sample buffer using dialysis, diafiltration or desalting chromatography.

Recommended Buffers

Adsorption buffer: 0.01 – 0.05 M phosphate or Tris-HCl (pH 6 to 9)

Elution buffer: 0.1 – 2.0 M sodium chloride in adsorption buffer.

Other common buffer systems may be used. For additional information on chromatographic methods of protein purification, see References.

Regeneration and equilibration

After separation, wash the binding material with over 5-bed volume of high ionic strength solution (1-2 M NaCl). After washing the column, feed the over 5-bed of the adsorption buffer, or until the column eluate will be stable pH and conductivity values.

Depyrogenation

The column wash with 5-bed volume of 0.2 M NaOH let stand for 16 hours, and then wash with endotoxin-free water or equilibration buffer.

• 0.2 M NaOH-20 % EtOH is more effective for endotoxin-free. Moreover 0.2 M NaOH-90 % EtOH can rapidly decrease LPS, at least contact for 2 hours.

Chemical and Physical Stability

Stable in:

Most salts (NaCl, $(NH_4)_2SO_4$, etc.), Alcohol (30 % (v/v) IPA, 70 % (v/v) EtOH), Urea (6 M) and Guanidine-HCl (6 M)

pH 2 to pH 12 (MAX Q-r & MAX Q-h) at 40 $^{\circ}$C, 1week

0.1M NaOH at less than 20°C, 4weeks (MAX Q-r & Q-h)

Cleaning-in-place (CIP)

Cellufine MAX Q performance remains constantly at least 100 CIP operating cycles with 0.5 M NaOH.

Flow Rate

Cellufine MAX Q-r and MAX Q-h are based on highly cross-linked cellulose gel, and have stability at high flow velocities.

1,000 cm/h in a 2.2 cm diameter column with 20 cm bed height at < 0.3 MPa.

Over 500 cm/h 30 cm diameter column with 20 cm bed height at < 0.3 MPa.

Storage

Store unopened container at ambient temperature. Do not freeze.

Short-term storage for bulk and column (2 weeks or less) can be at a room temperature with pH 2 to pH 13 (MAX Q-r and MAX Q-h). It is possible to store under alkaline condition at less than 20 °C as recommendation.

0.1 M NaOH ; MAX Q-h for 2 months, MAX Q-r for 2 months

0.5 M NaOH ; MAX Q-h for less than 1 week, MAX Q-r for 2 week

Longer storage should be in neutral buffer containing 20 % ethanol, at 2 – 8 °C. Do not freeze.

Shelf Lifetime

5 years from date of manufacture

References

1. Harris, E.L.V. and Angal, S., *Protein Purification Methods: A practical Approach*. New York: Oxford University Press, 1989.
2. Janson, J.-C. and Ryden, L., *Protein Purification: Principles, High Resolution Methods, and Applications.* 2nd ed. New York: John Wiley & Sons, Inc., 1998

Product Ordering Information (Catalogue No.)

Media type	Pack Size					
	MC* 1mL x 5	MC 5mL x 5	100 mL	500 mL	5 L	10 L
Cellufine MAX™ Q-r	20500-51	20500-55	20500	20501	20502	20503
Cellufine MAX™ Q-h	20600-51	20600-55	20600	20601	20602	20603

MC = Mini-Column

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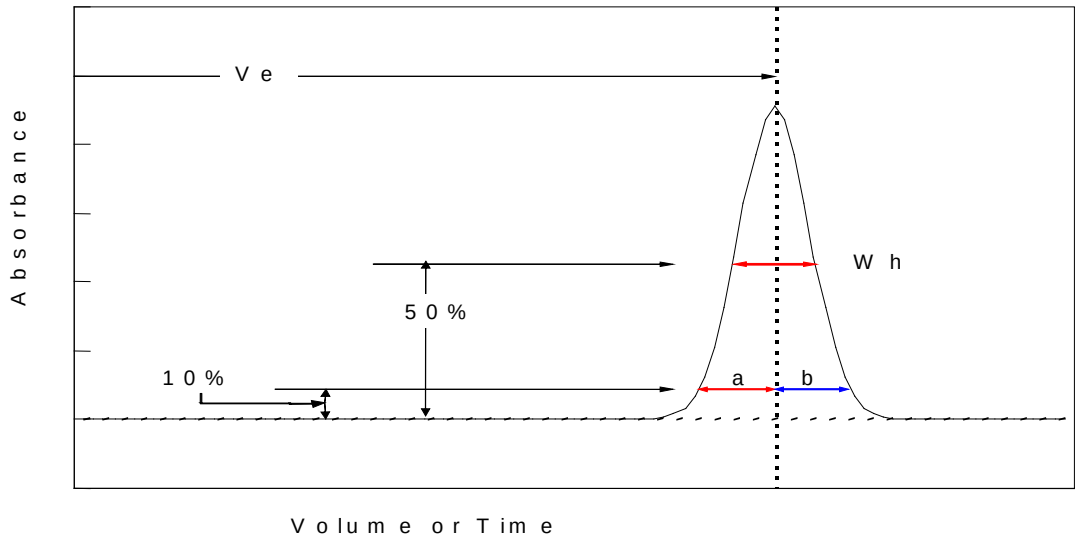
Appendix 1: Evaluation of column packing of Cellufine

Evaluation methods for the packing status of a column can employ indices, such as the number of (theoretical) plates (N), the height equivalents to a theoretical plate (HETP), and a symmetry factor (As).
The selected evaluation indices are affected by the measurement condition. For example, they vary with changes in the difference between the diameter/height of a column, the difference in piping, solvent sample volume, the flow velocity, temperature, etc.
Therefore, it is necessary to use the same measurement conditions each time.

Conditions	
Sample volume	1%（MAX 2.5%）of column bed volume
Sample concentration	1-2 % (V/V) acetone or 1M NaCl
Flow rate	~30 cm/h (X mL/hr/column cross section)
Detector	UV, conductivity



Formula

HETP = L/N

$N = 5.54 \times (Ve/Wh)^2$

L	Column length [cm or m]
Ve	Elution time or volume
Wh	Half of width of peak
a,b	Peak width of 10% peak hight (a)front, (b)rear
Note	Ve,Wh and a, b should have same dimensional units

(Note)
Generally, a larger value of N is good. (Likewise a smaller value of HETP is good.)
The asymmetry factor value (As) should be close to 1. Generally, acceptable symmetry values range from 0.8-1.6.

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8. **Agents, etc**. - No agent, employee or other representative has the right to modify or expand JNC Corporation's standard warranty applicable to the Products or to make any representations as to the Products other than those set forth in JNC Corporation's product literature and any such affirmation, representation or warranty, if made, should not be relied upon by Buyer and shall not form a part of this contract.

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12. **Governing Law** - This contract shall be governed by and construed in accordance with the laws (other than those relating to conflict of laws questions) of the Commonwealth of Japan.

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